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NEWS FROM AMRIS

THE ADVANCED MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY FACILITY AT THE UNIVERSITY OF FLORIDA

MR Microscopy in Alginate Beads as a Tool for Developing Artificial Organs

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In the field of tissue engineering, alginates have been employed extensively to encapsulate islets and cells (for example, references 1,2) that may then be implanted into humans, for example in an effort to develop a bioartificial pancreas. In this respect, the goal is to have the implanted alginate structures substitute pancreatic function, producing insulin in a controlled fashion for the treatment of diabetes. It is well known that different alginate structures support cell growth to differing degrees, but these properties have not been characterized. Generally, alginates with high guluronic acid content develop stiffer, more porous gels that maintain their integrity for longer periods of time and hinder cell growth after encapsulation. Conversely, alginates rich in mannuronic acid residues develop softer, less porous beads that tend to disintegrate more quickly, but cells encapsulated in these alginates tend to proliferate without inhibition. Additionally application of a poly-L-lysine layer surrounding the bead is used to provide mechanical support and immunoprotection.

The goal of our present studies is to use MR microscopy and microspectroscopy to characterize and monitor cell growth in individual alginate beads of different types, and with varied coating designed to control chemical exchange through the bead. The development of high field MR and microcoil technology makes suitable data collection feasible in realistic timescales with respect to cell viability. Drs. Constantinidis and Simpson performed feasibility studies at the NHMFL on the 600 MHz wide bore magnet as outside users from Emory University, but have for the last year been employed at UF, continuing their studies in the AMRIS facility.

To date four types of alginate have been analyzed: a high molecular weight, high guluronic acid content alginate (MVG); a lower molecular weight, high guluronic acid content alginate (LVG); a high molecular weight, high mannuronic acid content alginate (MVM); and a lower molecular weight, high mannuronic acid content alginate (LVM). In the bead preparation, a $CaCl_2$ solution is used to gel the alginate droplets in concentrations ranging

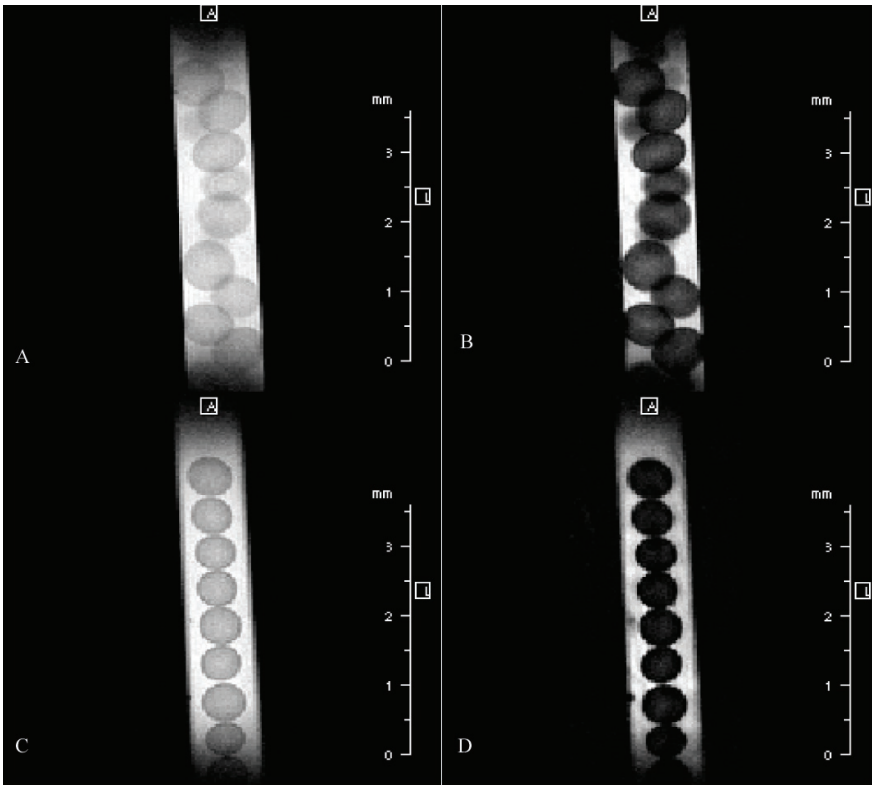
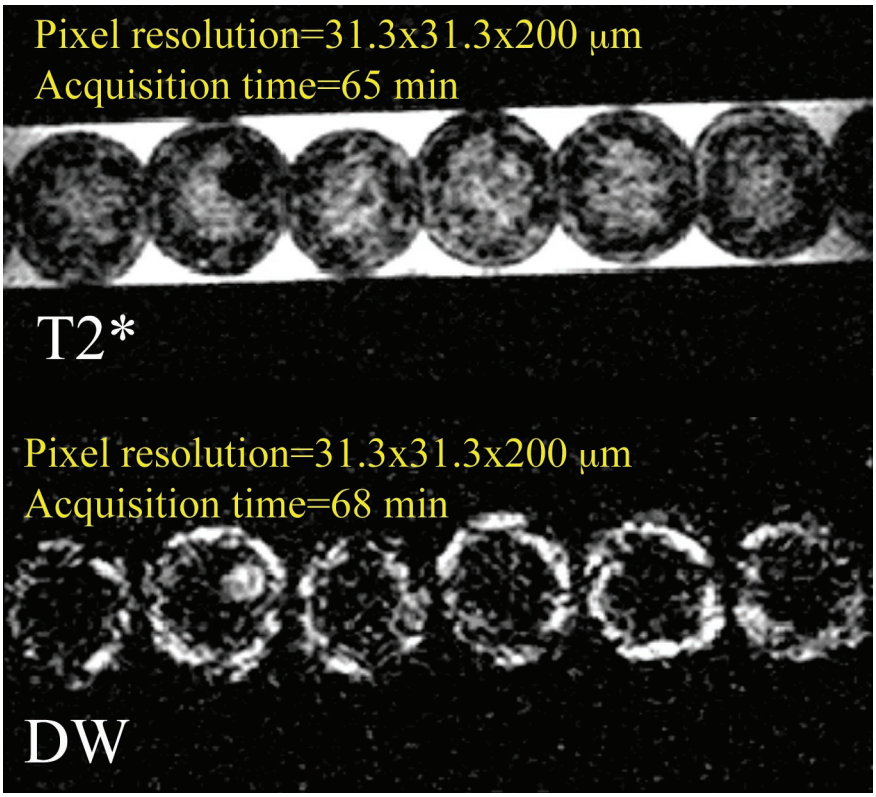


Figure 1: T2-weighted images for 2% LVG (top) and LVM (bottom) gelled with 100mM CaCl2. TE=15ms for A&C; TE=95ms for B&D. The darker signal from the LVM beads on the weighted images indicates a shorter T2.

10 to 100 mM. A few alginate beads were coated with poly-L-lysine (MW=19,200) to form alginate/poly-L-lysine/alginate (APA) beads in order to assess the effect of a PLL layer on the ability of alginate to re-gel in the presence of $CaCl_2$. NMR data were acquired using the 17.6 T Bruker wide bore system at UF. Alginate beads were loaded into a homebuilt, susceptibility-matched solenoidal microcoil having a diameter of 1.7 mm and length of 6 mm. T_2, T_1 and diffusion weighted image series yielded T_1, T_2 and ADC values using single bead ROI analysis.

The average T_1 of water in the beads showed no significant differences between alginates and only slight decreases compared to bulk water. The T_2, however, displays significant differences as a function of alginate and gellating Ca^{+2} concentration (see Figure 1). Higher concentrations of $CaCl_2$ result in a more tightly bound alginate matrix, while high guluronic content results in increased alginate order and structure. Because it is related more closely to the fraction of bound water, T_2 relaxation offers a good indicator of porosity and structure in the alginate bead. Interestingly, the addition of a PLL layer around the bead significantly alters the transverse relaxation of bead water by hindering the penetration of gellating Ca^{+2} into the liquefied core of the bead.

Transverse relaxation thus appears to be a valuable method of contrast in analyzing the structure and complexity of alginate gels used as a construct for encapsulating cells, and these data are being presented at a meeting[3] and have been submitted for publication[4]. Using T_2 methods, alginate structure and degradation can be monitored *in vitro* and *in vivo*, pre- and post-implantation. With regards to the construction of a bioartificial pancreas, these alginates have been used successfully to encapsulate islets and transformed cells. Initial studies at 17.6 T have shown that diffusion-weighted imaging is an excellent tool for monitoring the proliferation and distribution of these cells in alginate beads throughout the growth cycle. Figure 2 shows images of beads implanted with cells, clearly indicating the ability of the imaging to give and index of cell density and distribution. Additionally, preliminary results using spatially localized 1H spectroscopy indicate that the choline and lactate peaks may prove useful indicators of cell viability. By combining the imaging and spectroscopic information, it will be possible to monitor the viability and integrity of the entire construct noninvasively throughout the entire life of the artificial organ.



1 Stoke, B.T., *et al*., Small-angle X-ray scattering and rheological characterization of alginate gels, *Macromol. Symp.*, **120**, 91-101 (1997).

2 Constantinidis, I., *et al*., Effects of Alginate Composition on the Metabolic, Secretory, and Growth Characteristics of entrapped βTC3 Mouse Insulinoma Cells, *Biomaterials*, **20**, 1969-1975 (1999).

3 Grant, S.; Simpson, P; Blackband, S. and Constantinidis, I., Development of a Bioartificial Pancreas: High Resolution MR Analysis of Water Relaxation and Diffusion in Alginate Beads, ISMRM, Toronto, Ontario, Canada, May 2003.

4 Simpson, N.; Grant, S.; Blackband, S. and Constantinidis, I., NMR properties of alginate beads. Submitted, Biomaterials (2003).

Figure 2. bTC3 cells in beads made from 2% LVG alginate & gelled with 1.1% CaCl2. The bright signal on the diffusion scans (bottom) indicate the presence of cells, and are complimented by dark signal on the T2* weighted images (top).